ORIGINAL ARTICLE

Zinc, copper, selenium and manganese blood levels in preterm infants

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Objective: To measure the zinc, copper, selenium and manganese blood levels in a cohort of 68 preterm infants, and to establish any associations with growth and/or dietary intake.
Design: Blood samples were collected at an infant's expected date of delivery (term) and 6 months later. Serum zinc, plasma copper and whole blood manganese were analysed by atomic absorption spectrometry, plasma and red cell selenium were determined by mass spectrometry. Growth and dietary intake determinations have been previously published.
Setting: Hampshire, England.
Results: Mean (SD) birth weight of the infants was 1.47 (0.434) kg and mean gestation was 31.4 (2.9) weeks. Mean blood levels at term and 6 months were: serum zinc 12.0 (2.6) µmol/l and 13.8 (2.5) µmol/l; plasma copper 10.1 (2.6) µmol/l and 19.2 (3.6) µmol/l; plasma selenium 0.49 (0.15) µmol/l and 0.72 (0.14) µmol/l; red blood cell selenium 1.68 (0.40) µmol/l and 1.33 (0.19) µmol/l; and blood manganese 320 (189) nmol/l and 211 (68) nmol/l, respectively. There were no significant associations between levels of zinc and copper and dietary intakes of those nutrients at either age (dietary intakes of selenium and manganese were not determined). Only copper levels at term were significantly associated ($r = 0.31$; $p = 0.05$) with a growth parameter (head circumference).
Conclusion: These results provide new information about trace element status in this vulnerable population.

Trace elements are essential nutrients for metabolism, growth, and neurological and immunological function.[1–3] Zinc is an important micronutrient that supports normal growth.[2] Preterm infants are especially vulnerable to zinc deficiency.[4 5] Selenium is an essential component of selenoproteins including the antioxidant glutathione peroxidase. At birth, blood levels of selenium are lower in preterm infants than in term infants and continue to fall until weaning is commenced.[6–8] Copper is a cofactor in several metalloproteins, essential for oxidative metabolism, myelination and the metabolism of several steroid hormones.[2] Clinical copper deficiency is a recognised hazard among preterm infants.[9] Manganese is an essential micronutrient component of several enzymes including pyruvate carboxylase, mitochondrial superoxide dismutase and enzymatic systems of matrix turnover in skeletal growth.[10 11] The measurement of the variation in trace element blood levels in preterm infants throughout infancy, and the range of levels associated with good development are important areas of research.

We have previously reported a randomised clinical trial of a specially devised nutritional strategy in preterm infants.[12] The trial aimed to analyse blood mineral levels at term (expected date of delivery) and 6 months' gestation corrected age (GCA), and assess dietary intake and growth at these ages. As we did not find any significant differences in the blood levels of trace elements in the two arms of the trial, in this paper we report on the combined results of all infants enrolled in the trial to provide information on zinc, selenium, copper and manganese blood levels in a healthy population of UK preterm infants. The interaction between growth and dietary intake and blood biochemistry is also explored.

SUBJECTS AND METHODS

The study population comprised 68 preterm infants (35 boys, 33 girls), recruited in 1998 and 1999 to a randomised clinical trial conducted in Hampshire, UK. There were minor differences between the study group and the population nationally. There was a higher proportion of mothers aged over 30 years (55% vs 46%, respectively) and a larger proportion of first born infants (60% vs 47%, respectively) but the families participating in the study were of a similar social class distribution to that of the UK.[13 14] The Winchester local research ethics committee approved the study (reference number 003/97W).

The study methodology, including recruitment criteria, biochemical assays, growth determinations and dietary intake measurements, has been previously described.[12] Blood samples were collected at term ± 3 weeks and 6 months GCA ± 3 weeks by venepuncture, following the application of an amethocaine (Ametop, Smith & Nephew, UK) anaesthetic cream. All equipment was analysed for background zinc levels. We analysed plasma zinc and copper by flame atomic absorption spectrometry.[15] Selenium was measured in plasma and in red blood cells by inductively coupled plasma mass spectrometry and whole blood levels were calculated using haematocrit values.[16] Blood manganese was measured by atomic absorption spectrometry using electrothermal atomisation.[17]

Statistical analysis

The relevant biochemical data from the two comparative study groups was combined and analysed by appropriate observational and comparative statistical analysis. The mean or median values, standard deviations and ranges of values of biochemical variables are described. Student t tests were used to make paired and unpaired comparisons and to investigate correlation. In situations in which observations were clearly not normally distributed, t tests were replaced by corresponding rank-based non-parametric tests. Type 1 error probabilities at or below 5% have been judged significant.

Abbreviation: GCA, gestation corrected age

Table 1 Clinical information and nutritional support in the neonatal unit

	Number		
	Total	n (%)	Further information
Ventilation*	67	26 (39)	
Surfactant*	66	19 (29)	
Steroids*	66	39 (59)	Antenatal dexamethasone
Blood transfusion*	67	15 (22)	Median 40 ml, range 10–101 ml
Intraventricular haemorrhage	68	4 (6)	Grades I – IV[18]
Chronic lung disease	68	3 (4)	Oxygen supplementation >28 days
Seizures	68	1 (2)	
Total parenteral nutrition	68	20 (29)	Median number of days 4, range 2–18

*Full data were not available for two infants who were transferred between neonatal units.

RESULTS

Subjects

The mean (SD) birth weight of the 68 infants was 1.47 (0.434) kg (range 0.635–2.150 kg) and mean gestational age was 31.4 (2.9) weeks (range 25–36 weeks). Table 1 shows the clinical characteristics of the infants.

Diet

In the neonatal unit 64 (94%) infants were fed human milk or a mixture of human milk and an infant formula, 4 (6%) received only human milk and 4 (6%) received only infant formula(s). Among the infants who received human milk 45 (70%) had breast milk fortifier added for one or more days. Low birthweight formula was given to 91% of infants in the neonatal unit. Human milk was consumed by 27 (42%) infants at term and by 7 (11%) infants at 6 months GCA. Multivitamins, iron and folic acid were consumed by 66 (100%), 58 (88%) and 59 (89%) infants, respectively, at term and by 33 (54%), 21 (34%) and 14 (23%) infants, respectively, at 6 months GCA.

At term (first blood sample), human milk formed all or part of the diet for 27 (42%) infants and the mean (SD) dietary protein energy ratio was 8.9% (0.5%). Mean daily intakes of energy, iron, zinc, vitamin C and vitamin A were higher than the recommended daily intakes,[19] and mean daily intakes of protein, sodium, calcium and copper were lower than the recommended daily intakes.[19] At 6 months GCA (second blood sample), human milk formed part of the diet in 7 (11%) infants and the mean dietary protein energy ratio was 12.7% (2.2%). Mean daily intakes of energy, protein, sodium, iron, phosphorus and vitamin C were similar to recommendations but mean daily intakes of calcium, zinc and copper were lower than recommendations.[19]

Blood results

At the first blood test the mean postnatal age of the infants was 8.5 (3.3) weeks, 0.2 (1.4) weeks GCA. At the second blood test the mean postnatal age of the infants was 35.1 (3.4) weeks, 26.6 (1.7) weeks GCA.

At term and 6 months GCA, plasma zinc and selenium values were similar to published values for preterm or term infants.[20–23] At term, the mean plasma copper values were higher than published values for preterm infants reported by Aggett (converted means 7.6 and 8.2 μmol/l)[22]; but were comparable with reported results by Rajaram (converted mean 9.7 μmol/l).[24] At 6 months GCA, the mean plasma copper values reflected published values.[22 24] At term and at 6 months GCA, the mean and median manganese values were similar to published values (converted range 255–309 nmol/l) for term infants in their first year (table 2).[22]

By 6 months GCA, the range of values for zinc, selenium and manganese had narrowed whereas the range of values of plasma copper had widened. The mean plasma levels of zinc, selenium and copper increased significantly whereas mean red blood cell selenium and blood manganese levels had decreased significantly. With the exception of plasma selenium, the mean values of blood analytes in infants who had received blood transfusions ($n = 15$) and those who had not ($n = 52$) did not differ significantly. Infants who had received transfusions had a significantly lower mean plasma selenium levels (0.39 μmol/l and 0.51 μmol/l, respectively; $p = 0.008$). Similarly, infants who had received parenteral nutrition had a significantly lower mean selenium plasma value than infants who had not (0.42 μmol/l and 0.52 μmol/l, respectively; $p = 0.02$). The effects of blood transfusion and parenteral nutrition had disappeared by 6 months GCA.

There were no significant gender differences in the blood analytes with the exception of mean whole blood selenium at 0 months GCA and red blood cell selenium at 6 months GCA, which were significantly higher in females (table 3).

Interactions

We did not find any significant associations between the dietary intakes of zinc and copper and the corresponding blood analytes at term or 6 months GCA. It was not possible to

Table 2 Results of trace element analysis at term and at 6 months gestation corrected age (GCA)

		Observed values			
Analyte	Time of sampling	Mean (SD)	Median	Range	n
Plasma zinc, μmol/l	At term	12.0 (2.6)	11.8	5.0–20.5	58
	At 6 months GCA	13.8 (2.5)*	13.2	9.1–22.7	54
Plasma copper, μmol/l	At term	10.1 (2.6)	9.4	5.8–17.0	51
	At 6 months GCA	19.2 (3.6)*	19.1	10.5–26.4	56
Plasma selenium, μmol/l	At term	0.49 (0.15)	0.49	0.21–0.88	62
	At 6 months GCA	0.72 (0.14)*	0.73	0.49–1.09	56
Whole blood selenium, μmol/l	At term	0.87 (0.21)	0.83	0.48–1.49	56
	At 6 months GCA	0.94 (0.12)	0.95	0.68–1.23	50
Red cell selenium, μmol/l	At term	1.68 (0.40)	1.67	0.7–2.55	56
	At 6 months GCA	1.33 (0.19)*	1.31	0.92–1.89	51
Whole blood manganese, nmol/l	At term	320 (189)	281	110–1129	54
	At 6 months GCA	211 (68)†	202	86–400	50

*$p = 0.001$, †$p = 0.002$, second vs first test.

Table 3 Selenium status (mean (SD)) of boys and girls

Selenium	Time of sampling	Boys	Girls
Plasma selenium, µmol/l	At term	0.47 (0.13)	0.51 (0.17)
	At 6 months GCA	0.70 (0.12)	0.75 (0.15)
Red blood cell selenium, µmol/l	At term	1.59 (0.41)	1.78 (0.37)
	At 6 months GCA	1.27 (0.16)*	1.38 (0.21)
Whole blood selenium, µmol/l	At term	0.80 (0.18)†	0.93 (0.23)
	At 6 months GCA	0.91 (0.10)	0.97 (0.14)

GCA, gestation corrected age.
*p = 0.04, †p = 0.02, boys vs girls.

determine selenium or manganese dietary intakes with accuracy at either assessment point.

There were no significant associations between height, weight and head circumference with trace element levels at either sampling with the exception of plasma copper at term which was weakly correlated with head circumference ($r = 0.31$; $p = 0.05$).

DISCUSSION

Published biochemical reference data on trace element status in low birthweight infants is limited and needs updating for use in the clinical setting; that which is published indicates that preterm infants routinely have low stores of essential minerals such as zinc, copper, selenium and manganese. In this paper, the blood levels of the trace elements zinc, selenium, manganese and copper are reported at two points in infancy.

Our study population was generally representative of preterm infants in the UK. Only 4% of study families chose not to complete the study and their characteristics were typical of the study population. The initial human milk feeding rate (94%) among the study infants was higher than the nationally reported rate (71%) for full term infants,[13] but comparable with that reported (84%) in a recent survey of low birthweight feeding practices.[25] Even so, the high initial human milk feeding rate in our study may not be typical of UK preterm infants. Thus, some caution may be needed in using these trace elements levels as norms for other populations of preterm infants.

Most of the blood tests (68%) were performed within the time period of $\pm$1.4 weeks of term or 6 months GCA and all were performed within 3 weeks of the specified age.

Blood levels

Clinical interventions in the neonatal unit had no observable effects on blood trace element analyses, with the exception of plasma selenium levels at term, which were lower in infants who had received blood transfusions or who had received total parenteral nutrition than in the rest of the cohort. The use of plasma depleted red cells in current transfusions and/or the babies receiving blood transfusions or total parenteral nutrition (TPN) being among the most premature could account for this effect. These differences were transitory and had disappeared by 6 months GCA.

We found a wide range of values of all analytes at term. This may be a reflection of the heterogeneous population (wide range of birth weights, gestational ages and severity of postnatal illness) in the study, or of variations in intrauterine nutrient accretion rates, or a combination of factors. By 6 months GCA, the range of values of analytes, with the exception of copper, was narrower than at term indicating that the effects of the intrauterine environment and early postnatal complications had dissipated. In addition, mean values of plasma zinc, selenium and copper increased significantly by the time of the second blood test, indicative of improved body stores. It is not clear why the range of copper values increased between term and 6 months GCA—it could be the result of variations in environmental exposure to copper.

Our observations of a decrease in blood manganese levels between term and 6 months GCA are in accord with other published values.[22 26] High blood manganese levels around birth result from in utero accumulation.[26 27] The subsequent decrease in blood manganese levels with age is believed to indicate the gradual movement of manganese from the blood to tissue storage. Hence, the transition in the manganese blood:tissue partition gave rise to lower manganese values for the study infants at 6 months GCA compared with those at term.

The mean values of plasma zinc were comparable with published values for preterm infants at both test points whereas the mean plasma copper value (10.1 µmol/l) was higher than the reference value at term.[22] The difference between the mean copper level of the subjects at term and previously published values might have been due to the recent increase in copper content of infant milk formulations in line with the infant formula and follow-on formula regulations and/or the variation in the copper content of water supplies with geographical location.[28] The mean plasma levels of selenium at term and 6 months GCA fall within the normal ranges for infants of the same GCA.[29] In general, any lack of comparability between study values and previously published data may be explained by changes in the nutritional management of preterm infants in recent years or by differences in cohort characteristics.

We did not find any gender differences in blood results at term or 6 months GCA, with the exception of a significantly higher mean whole blood selenium level, at term, and a significantly higher mean red blood cell selenium level, at 6 months GCA, in girls than in boys. Two measures of selenium status, Se-dependent glutathione peroxidase activity and erythrocyte selenium, have previously been found to be higher in female compared with male preterm infants.[30 31] Red blood cell and whole blood selenium levels are indicative of selenium status over a longer time than plasma selenium values.[22] Thus, it could be hypothesised that boys have a higher physiological requirement for selenium than girls, such that plasma selenium levels, which reflected current nutrient intakes, were similar for both sexes but the whole and red blood cell selenium levels were lower for boys because of greater metabolic need. Alternatively, preterm girls may begin life with better selenium stores than preterm boys.

Interactions

We found no associations between the trace element levels in blood and dietary intakes of protein, iron, zinc and copper at term or 6 months GCA and only one association between growth and trace element blood levels. This lack of association may be due to the wide ranges of both micronutrient intakes and the trace element blood levels, or due to not all infants having achieved metabolic equilibrium by term or that preterm infants may have marginal body stores of trace element minerals during infancy. In addition, at term, the infants' mean intake of copper was below the current recommendation and would probably not increase body stores,[19] the strong homoeostatic control of body zinc status renders a correlation between blood levels and zinc intakes unlikely, except at extremes of intake, and for formula-fed infants the copper content of the water supply may be the main determinant of copper status. Hence, the lack of an association between mineral intakes from food and milk, and plasma mineral levels is not surprising.

The association between copper intake at term and head circumference is not readily explained. This may be a chance finding or it is possible that copper may be the limiting nutrient for brain enlargement at this point but not for somatic growth.

What is already known on this topic

Trace elements are important for the normal growth and development of preterm infants.

What this study adds

Information is presented on the levels of plasma zinc, plasma copper, plasma selenium, red cell selenium, whole blood selenium, and blood manganese in preterm infants at the time of the estimated date of delivery and 6 months later.

Further research is needed to elucidate the complex interactions between nutrition and brain development

CONCLUSION

The English preterm infant cohort in this unique study has been very well described, and all assessments were completed within tight deadlines to provide blood analyses at specific ages. We have described the levels of plasma zinc, plasma selenium, red cell selenium, whole blood selenium, plasma copper and blood manganese for preterms at their expected date of delivery ± 3 weeks and at 6 months GCA ± 3 weeks. These results provide important, previously unpublished information about trace element status in this vulnerable population.

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